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13. Abstract ***(Maximum 200 Words)*** *(abstract should contain no proprietary or confidential information)*

Previous analyses of C/EBPß KO mice have demonstrated an important role for this transcription factor in early developmental events in the mammary gland. In the pre-pubescent mouse, progesterone receptor (PR) expression is uniform along the ducts, but becomes non-uniform following the completion of ductal morphogenesis. However, PR remained uniformly expressed in the C/EBPß KO glands. Similar increases in prolactin receptor (PrlR) and estrogen receptor were also observed. When PR expression was examined in the PrlR KO gland, a uniform pattern of expression was also observed. In order to determine if these effects were epithelial cell autonomous and independent of any alterations in systemic hormone levels, PrlR KO and Stat5A/B double KO mammary glands were transplanted into the cleared fat pads of nude mice. Quantitation of PR- and BrdU-positive cells in the glands of Stat5A/B KO transplants showed levels intermediate to that of wildtype and PrlR KO, suggesting that not all of the PrlR signaling is mediated through Stat5. Proliferating ductal cells rarely co-localized with steroid receptor-positive cells, supporting a paracrine mechanism of action. Together these data suggest that C/EBPß regulates mammary epithelial cell fate resulting in the correct spatial pattern of gene expression required to permit steroid hormone-regulated cell proliferation.

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• Introduction

The study of mouse knockout (KO) models has helped elucidate the in vivo function of many different genes involved in the various stages of mammary gland development. Previous analyses of C/EBPß KO mice have demonstrated an important role for this transcription factor in ductal morphogenesis and lobouloalveolar development of the mammary gland, and can be used as a powerful tool to examine the development of the mouse mammary gland. The correlation of increased PR expression coupled with an inhibition of proliferation in this mouse model provides a novel way of viewing PR signaling events. By identifying genes involved in the normal development of the mammary gland, we may be able to find new targets for the treatment of breast cancer.

• Body

Objective 1: *Investigate the potential role of known genes that may mediate the paracrine action of PR required for alveolar proliferation using the C/EBPß-/- mouse model.*
Objective 2: *Identify and characterize novel downstream gene targets differentially expressed in the C/EBPß-/- mammary gland.*

1A • *Isolate total RNA from virgin and E+P treated mammary glands from C/EBPß wildtype and knockout mice*
• *Construct riboprobe vectors*
• *Perform RPA and Northern analyses*

Mammary glands have been collected from wildtype and C/EBPß knockout mice, from both virgin animals and from those treated with estrogen and progesterone (E+P) for two days. Some of the tissue was used to isolate RNA, and the rest of the tissue was either fixed in paraformaldehyde, embedded in paraffin and sectioned for tasks 1B and 1C or frozen in liquid nitrogen and used to make RIPA whole cell extracts for Western blot and immunoprecipitation analyses. More tissue will be collected as needed.

I now have a number of riboprobe vectors for use in ribonuclease protection assays (RPA) or Northern analyses, eleven of which I generated myself. Some of the probes have been used for RPA and Northern blot analyses, and these analyses are still being performed. To date, I have shown that progesterone receptor (PR), prolactin receptor (PrlR), estrogen receptor (ER) and p27 (cyclin-dependent kinase inhibitor) mRNA expression is increased in the C/EBPß KO mammary gland as compared to the wildtype. Another gene whose expression is increased in the C/EBPß KO mammary gland is a small proline-rich protein (SPRR2A) that is normally expressed in the skin. This is an example of a gene identified from our suppressive subtractive hybridization (SSH) screen of differentially expressed genes in Task 2A. Based on this finding, we are investigating the possibility that other skin genes may be expressed in the C/EBPß KO mammary gland. I have obtained riboprobe vectors and antibodies to some candidate genes, such as involucrin and loricrin. There are also genes that are expressed at lower levels in the C/EBPß KO, such as insulin-like growth

factor binding protein 5 (IGFBP5), calcitonin, and insulin receptor substrates 1 and 2 (IRS-1, IRS-2).

1B • *Perform in situ hybridization on paraffin-embedded mammary gland sections, comparing WT and KO, using riboprobes from above*

A limited number of *in situ* hybridization analyses have been perfomed in our lab, due to technical difficulties. Low levels of gene expression also make detection difficult. We are now optimizing a new protocol that does not use radioactivity and emulsion/silver grains for detection, but instead is a more sensitive, non-radioactive method and uses an alkaline phophatase/Fast Red stain to detect hybridization. One other change we can make is to use fresh frozen sections rather than fixed and embedded tissue.

In collaboration with Terri Wood at Penn State University, we have performed *in situ* hybridization for IGFBP5 on frozen sections. The levels of IGFBP5 mRNA not only decreases in the C/EBPß KO mammary gland, but the pattern of expression changes as well. IGFBP5 is expressed uniformly in almost every epithelial cell in the ducts of WT mammary glands, but the pattern of expression became punctate, with staining in only certain epithelial cells in the KO gland. While we observed a decrease in total IGFBP5 message levels by Northern analysis, the change in patterning could only be visualized by *in situ* hybridization. We have also examined the expression of other members of the IGF axis, such as IGF-IR and IGF-II, and have seen no difference in expression.

Similarly, a collaboration with Russ Hovey and Barbara Vonderhaar at the NIH has yielded nice *in situ* hybridization results using PR and PrlR probes on paraffin-embedded tissue sections. As we have previously shown for PR protein by immunostaining, PR mRNA expression is scattered along the ducts of WT glands but increases and becomes uniform in the C/EBPß KO glands. The same is true for PrlR mRNA expression; there is punctate expression in the WT virgin glands that becomes uniform in the C/EBPß KO glands.

1C • *Obtain antibodies to candidate proteins and perform immunohistochemistry.*

We have previously shown that PR protein expression restricted to a sub-population of ductal epithelial cells in the WT virgin mammary gland. The PR-positive cells rarely co-localize with proliferating cells, as determined by double immunofluorescence staining for PR and BrdU-incorporation. In order to define the normal pattern of PR staining during ductal development, we analyzed mammary glands from wildtype C57Bl/6 mice at 6, 9, and 12 weeks of age. We made the interesting observation that PR expression is uniform at 6 weeks and protein expression is downregulated between 9 and 12 weeks to give the scattered expression pattern we had seen in mature virgin glands. Thus, it is lack of downregulation of PR rather than an increase in expression that occurs in the C/EBPß KO.

Because the mammary gland phenotype of the C/EBPß KO mouse is very similar to the phenotype of the PrlR KO mouse, I examined the level and pattern of PR staining in PrlR KO mammary gland sections. Consistent with our results with the C/EBPß KO, the number of PR-positive ductal epithelial cells was three-fold greater in the PrlR KO as compared to wildtype. Associated with this higher level

of PR was a seven-fold decrease in proliferation after treatment for 2 days with E+P, as determined by BrdU incorporation. I also analyzed and quantitated PR staining during ductal development at 6, 9, and 12 weeks in both wildtype and PrlR KO glands. As we found for the C57Bl/6 glands, PR levels are similar between the WT and KO at 6 weeks, but PR levels remain high in the KO at 9 and 12 weeks instead of decreasing during normal ductal development.

One of the concerns with studying the mammary glands in these various knockout models is that these mice also often have ovarian defects along with the mammary gland phenotypes. To rule out any systemic hormonal deficiencies and to prove the mammary gland phenotype is restricted to the epithelial cells, pieces of mammary glands from KO mice were transplanted into the cleared fat pads of immuno-compromised nude mouse recipients. As controls, wildtype glands were also transplanted and an endogenous gland from the recipient was removed and analyzed along with the transplants. Stat5 is a transcription factor that is directly downstream of prolactin signaling and exists as two isoforms: A and B. A knockout mouse has been created that deletes both forms, and glands from these mice were transplanted along with PrlR KO glands. Quantitation of PR- and BrdU-positive cells in the glands of Stat5 A/B double KO transplants showed levels intermediate to that of wildtype and PrlR KO, suggesting that not all of the PrlR signaling is mediated through Stat5. Future immunostaining experiements will now include sections from the Stat5 and PrlR KO glands to compare to the C/EBPß KO.

Additional immunostaining has been performed on C/EBPß KO mammary tissue using antibodies against p27, estrogen receptor (ER), and phospho-MAP kinase. Preliminary results suggest that both p27 and ER expression increases in the C/EBPß KO, but this data has not been quantitated. I have optimized immunostaining against the phosphorylated form of MAP kinase, but the levels of protein are too low even in the WT glands to quantitate.

Finally, immunostaining against the skin protein SPRR2A, which is overexpressed in the C/EBPß KO mammary gland, has been performed. As predicted by Northern blot analysis results, there is no detectable SPRR2A staining in WT ducts, but significant signal is seen on the apical surface of KO ducts.

2A • *Screen KO tester and WT tester libraries for additional differentially expressed partial cDNA clones*
• *Confirm change in gene expression of SSH-PCR clones by Northern*
• *Construct normalized midpregnant mouse mammary gland cDNA library*

Suppressive subtractive hybridization (SSH) was performed to identify genes that are preferentially expressed in either the WT or C/EBPß KO mammary glands. We performed SSH using either virgin mammary glands or glands treated with E+P for 21 days. Genes more highly expressed in the WT are found in the KOsub library and genes with higher expression in the C/EBPß KO are in the WTsub library. Our more recent findings that PR expression is changing early in mammary gland development have led us to focus more heavily on the virgin SSH libraries. Appoximately 40 genes have been sequenced from the virgin WTsub and KOsub libraries. About half the genes were known and the other half were ESTs (expressed sequence tags) or unique genes. The clones that

matched ESTs are listed in a table in Appendix I and the tissue distribution of the ESTs is given. Half of the clones/ESTs were expressed in mammary tissues, which is a good indication that they will truly be differentially expressed in the C/EBPß KO mammary gland.

The virgin libraries will continue to be screened for additional genes to sequence and the resulting clones will be used in Northern blot analysis to confirm their differential expression. WTsub clone F51 was found to be upregulated in the C/EBPß KO by Northern and was shown to be homologous to the small proline-rich protein SPRR2A, discussed in the results for Objective 1. Two other clones, WTsub F28 and WTsub F80, are homologous to ESTs and were also confirmed by Northern blot analysis to be upregulated in the KO. Screening of a cDNA library for the full-length F28 gene has been performed (discussed below) and clone F80 is an excellent candidate to isolate as well.

Because of the focus on events in early mammary gland development, a virgin cDNA library was generated instead of a mid-pregnant mammary gland cDNA library. PolyA+ RNA was isolated from the mammary glands of mature virgin C57Bl/6 mice and 5 µg was used to reverse transcribe into cDNA. Linkers were added to the ends of the cDNAs and after digestion with EcoRI and XhoI, the cDNAs were directionally cloned into the Zap Express vector from Stratagene. The cDNA library was then packaged into phage and plated for screening. Aliquots of the library were frozen at –80°C.

2B • *Screen cDNA library for full length cDNA clones*
• *Sequence analysis of full length cDNAs*

Based on the EST distribution and the confirmation of overexpression in the C/EBPß KO gland by Northern blot analysis, WTsub clone F28 was selected to screen the virgin mammary gland cDNA library to isolate a full-length cDNA. Approximately $9x10^5$ cDNA clones were plated for the initial screen and after performing three rounds of screening 8 clones were isolated. There were three different size inserts ranging from 1.2 to 4.3 kilobases, and after sequencing it was found that all three inserts had a common 3′ end. However, Northern blot analysis shows that clone F28 corresponds to a cDNA of approximately 8 Kilobases in size, so only a partial F28 cDNA was isolated.

A BLAST search with the 4.3 Kb of F28 sequence did not pull up an exact match from the GenBank database, but did show limited homology with a gene found in mouse (CRP-ductin), rat (Ebnerin), rabbit (Hensin) and human (DMBT1). Sequence analysis of the F28 cDNA revealed one, large open reading frame (ORF) that contained consensus motifs for scavenger receptor cysteine-rich (SRCR) and zona pellucida (ZP) domains, which are also found in the CRP-ductin gene family. Based on the sequence similarity, but NOT identity, and the conserved domain structure it appears I have cloned a novel member of this gene family. The exact function of these genes has not been elucidated, but they do appear to be expressed in epithelial cells and may be involved with cell polarity, proliferation and differentiation. DMBT1 is located on human chromosome 10q25, a site that is frequently deleted in malignant brain tumors (which is how DMBT got its name).

- It will be necessary to isolate the 5′ end of the F28 gene, which I will accomplish by performing 5′ RACE (rapid amplification of cDNA ends) using a primer that hybridizes to the 5′ end of the known F28 sequence.

• Key Research Accomplishments

• Identification of a number of genes whose expression levels and patterns change in the C/EBPß KO mammary gland using a combination of RPA, Northern blot, *in situ* hybridization, Western blot and immunostaining analyses.

• Progesterone receptor expression is increased and proliferation is decreased in two other mouse knockout models: Prolactin receptor KO and Stat5A/B double KO.

• Construction of virgin mammary gland cDNA library from C57Bl/6 mouse strain completed.

• Isolation and sequencing of partial cDNA for a novel gene (designated F28) has been completed. Domain mapping and exon boundary mapping are complete for the 3′ end of the gene. 5′ RACE underway to isolate the 5′ end of gene.

•Reportable Outcomes

Abstracts: Refer to Appendix II
•Gordon Conference on Mammary Gland Biology, Barga, Italy (May 2000)
•International Congress of Endocrinology Meeting, Sydney, Australia (Oct. 2000)
•Hormones and Cancer Meeting, Port Douglas, Australia (November 2000)
•Molecular Mechanisms of Apoptosis, Keystone, Colorado (January 2001)
•Endocrine Society Meeting, Denver, Colorado (June 2001)

Presentations:
•Gordon Conference on Mammary Gland Biology, Barga, Italy (May 2000)
ORAL presentation
•Endocrine Society Meeting, Denver, Colorado (June 2001).
Poster presentation

Manuscripts:
•Signal Transducer and Activator Transcription 5 (Stat5) controls the specification of mammary alveolar epithelium. Keiko Miyoshi, Jonathan Shillingford, Gilbert H. Smith, Sandra L. Grimm, Kay-Uwe Wagner, Takami Oka, Jeffrey M. Rosen, Gertraud W. Robinson and Lothar Hennighausen. **Submitted** to the Journal of Cell Biology in July 2001.

Funding applied for, based on work supported by this award:
We have used the work presented in this report to apply for a competitive renewal of NIH grant CA16303. If awarded, funding would be used to supplement costs associated with this project.

•Conclusions

By comparing multiple mouse knockout models with similar mammary gland phenotypes, I hope to be able to pinpoint changes in gene expression that are responsible for, or markers of, the lack of lobuloalveolar development in these mice. So far, we have observed changes in the expression of a number of genes that are known to influence mammary gland development. However, the genes identified from SSH analysis may give the most insight into this process. Preliminary results indicate that it may not be a block in lobuloalveolar development, per se, but an alteration in cell fate at an earlier stage that may be responsible for the phenotype we see. Supporting this idea is the overexpression of SPRR2A, a marker of skin differentiation, in the C/EBPß KO mammary gland. The approach we are taking should be able to identify genes involved in the normal development of the mammary gland, and as a result we may be able to find new targets for the treatment of breast cancer.

Appendix I: Tissue distribution of EST hits from SSH screening

Library	Clone	Tissues	Best Hit
KOsub	A79	Mouse embryo, thymus	22/24 (91%)
KOsub	C40	Bovine pooled tissue, Human skeletal muscle	217/263 (82%)
KOsub	C45	Mouse brain, lactating mammary gland, embryo	215/221 (97%)
KOsub	C61	Pig pooled tissue, Human uterus and colon	51/58 (87%)
KOsub	C62	Mouse kidney	22/24 (91%)
KOsub	D5	Mouse skin, Rat kidney, Human ovary and cervix	340/342 (99%)
KOsub	D26	Human skeletal muscle and ovarian tumor, Mouse lung tumor	18/18 (100%)
KOsub	D42	Mouse craniofacial (e8.5), thymus, embryo, 4 wk mammary gland, blastocyst	429/431 (99%)
KOsub	D49	Mouse brain, pituitary, myotubes, blastocyst, eye, embryo, colon, lung tumor	380/400 (95%)
KOsub	D52	Mouse mammary tumor, hypothalamus, spleen, T cell, B cell, liver, lactating mammary gland	307/310 (99%)
KOsub	D59	Mouse lung tumor, e12 gonad, embryo, colon, mammary tumor, Human fetal liver, Xenopus embryo	245/250 (98%)
KOsub	FA12	Mouse kidney, Human melanoma and marrow	178/206 (86%)
KOsub	FA16	Mouse 4 wk mammary gland, mammary tumor, embryo, thymus, skin, salivary gland	399/406 (98%)
KOsub	FA31	Human brain, Mouse liver, mandible, B cell	20/20 (100%)
KOsub	FA72	Mouse mammary tumor, heart, brain, T cell, 4 wk mammary gland, embryo	436/451 (96%)
KOsub	FA78	Mouse mammary tumor, lung tumor, newborn skin, small intestine, Bovine embryo, Human hypothalamus, cervical carcinoma	410/423 (96%)
KOsub	J56	Alfalfa leaf/stem, Mouse lung tumor, craniofacial (e8.5)	34/38 (89%)

WTsub	E36	Mouse liver, lactating mammary gland, brain, lung, 4 wk mammary gland, kidney, Human testis, Bovine pooled mammary gland	452/455 (99%)
WTsub	E56	Mouse 4 wk mammary gland, newborn head, embryo	231/235 (98%)
WTsub	E79	Mouse brain, stomach, lactating mammary gland, colon, liver, ovary, uterus, pancreas, heart, kidney, embryo, tongue, testis	249/268 (92%)
WTsub	F28	Mouse thymus, heart, bladder, epididymus, neonate head, retina, pancreas	514/514 (100%)
WTsub	F80	Mouse brain, 4 wk mammary gland, mammary tumor, myotubes, Rat brain	417/422 (98%)

Appendix II: Meeting Abstracts

1. Gordon Conference on Mammary Gland Biology
Barga, Italy
May 21-25, 2000

Title: C/EBPß Controls Cell Fate Determination During Mammary Gland Development

Authors: SANDRA L. GRIMM*, Tiffany N. Seagroves*, Russell C. Hovey+, Barbara K. Vonderhaar+, Teresa L. Wood# and Jeffrey M. Rosen*

Affiliations: *Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX, +Laboratory of Tumor Immunology and Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD, and #Department of Neuroscience and Anatomy, Penn State University, Hershey, PA.

Abstract: Previous analysis of C/EBPß-deficient mice has demonstrated an important role for this transcription factor in ductal morphogenesis and lobouloalveolar development of the mammary gland. During normal ductal outgrowth progesterone receptor (PR) expression is uniform, but becomes non-uniform and scattered when the ductal morphogenesis is complete. However, PR protein and mRNA remained uniformly expressed and at higher levels in the C/EBPß KO gland. These changes were not restricted to PR; increases in prolactin receptor mRNA and the estrogen receptor were also observed. Additionally, differentially expressed clones, comprising both known and novel genes, have been identified using SSH-PCR. Surprisingly, SPRR2A, a marker of epidermal differentiation, and keratin 6 were found to be upregulated in the virgin KO gland suggesting a switch in the epithelial cell fate to a more epidermal lineage. These changes in gene expression were accompanied by a 10-fold decrease in epithelial cell proliferation. Proliferating ductal cells rarely co-localized with steroid receptor-positive cells, supporting a potential juxtacrine mechanism of action. Interestingly, a decrease in both IGF-II and IGFBP-5 has also been observed in the C/EBPß KO mice. Together these data suggest that C/EBPß regulates mammary epithelial cell fate resulting in the correct spatial pattern of gene expression required to permit steroid hormone-regulated cell proliferation (Supported by grants CA16303 and DK07696).

2. 11th International Congress of Endocrinology Sydney, Australia October 29 - November 2, 2000

Mammary gland development and oncogenesis

Rosen, J.M., Wyszomierski, S.L., Grimm, S. Contreras, A. and Medina, D

Studies of normal mammary gland(MG) development using transgenic and knockout mouse models have provided some new insights into the mechanisms regulating MG growth and differentiation, and alterations that occur in breast cancer. Composite response elements in the ß-casein promoter, a marker of MG functional differentiation, integrate prolactin- and glucocorticoid-regulated signal transduction pathways via protein-protein and protein-DNA interactions. Specific interactions of the glucocorticoid receptor with both STAT5 and C/EBPß are required for maximal casein gene expression. Both transcriptionally active and dominant negative isoforms of C/EBPß are expressed during normal mammary gland development and altered expression of these isoforms occurs in breast cancer. Knockout(KO) of C/EBPß results in altered ductal mophogenesis, decreased lobuloalveolar development and a lack of functional differentiation in the MG. C/EBPß appears to regulate mammary epithelial cell (MEC) fate resulting in the correct spatial pattern of gene expression required to permit steroid hormone regulated cell proliferation. Alterations in the distribution of the progesterone(PR) and prolactin receptors, as well as in the level of growth factors possibly involved in a paracrine regulation of cell proliferation are observed in the MECs of C/EBPß KO mice. In addition, the distribution of PR changes in early hyperplastic lesions observed in p53 null BALB/c MEC transplants, suggesting the possible change from a paracrine to an autocrine signaling pathway may occur in the transition from normal to premalignant lesions. Supported by grant CA16303 from the National Institutes of Health.

3. Hormones and Cancer 2000
Port Douglas, Queensland, Australia
November 3-7, 2000

Mammary Gland Development and Breast Cancer: Insights from Transgenic and Knockout Mouse Models

J.M. Rosen, S.L. Grimm, A. Contreras, D. Medina, K.L. Murphy, C.J. Ormandy, R.C. Hovey, B.K. Vonderhaar, T.L. Wood

Studies of normal mammary gland (MG) development using transgenic and knockout (KO) mouse models have provided some new insights into the mechanisms regulating mammary gland growth and differentiation, and alterations that occur in breast cancer. Gene deletion of the transcription factor C/EBPß, but not C/EBPalpha results in altered ductal morphogenesis, decreased lobuloalveolar development and a lack of functional differentiation in the mammary gland. C/EBPß appears to regulate mammary epithelial cell (MEC) fate resulting in the correct spatial pattern of gene expression required to permit steroid hormone regulated cell proliferation. A non-uniform pattern of progesterone receptor (PR) distribution established during ductal morphogenesis is required to permit the correct proliferative response to steroid hormones observed during pregnancy. Alterations in the distribution of PR and prolactin receptors (PrlR), as well as in the level of growth factors, such as IGF-II and their binding proteins are observed in the MECs of C/EBPß KO mice. In addition, the spatial pattern of PR expression is altered in PRLR and Stat5A& B KO mice, which also fail to undergo lobuloalveolar proliferation. Thus, there appears to be co-association and co-regulation of PR and PRLR during mammary gland development. In wild type mice, PR- and bromodeoxyuridine-positive cells are adjacent to each other and rarely co-localize. This distribution of PR is altered in hyperplastic lesions observed in p53 null BALB/c MEC transplants, suggesting the possible change from a paracrine to an autocrine signaling pathway may occur in the transition from normal to premalignant lesions. Finally, transgenic mice expressing a novel ligand-independent, drug-inducible FGFR have been developed to help elucidate the mechanisms by which local growth factors regulate mammary gland development. Supported by NIH grant CA16303.

4. Keystone Meeting "Molecular Mechanisms of Apoptosis" Keystone, Colorado January 16-22, 2001

Unique and redundant roles of Stats 5a and 5b in the proliferation and differentiation of mammary epithelial cells

Keiko Miyoshi [1], Sandy Grimm[2], Kay-Uwe Wagner [1,3], Gertraud W. Robinson[1], Jeffrey Rosen[2], and Lothar Hennighausen[1]

[1]Laboratory of Genetics and Physiology, NIDDK, NIH, Bethesda, MD, U.S.A. 20892, [2]Baylor College of Medicine, Houston, TX 77030, [3]University of Nebraska Medical Center, Omaha, NE, U.S.A. 68198

Functional development of the mammary gland proceeds in cycles and is controlled by steroid and peptide hormones. While estrogen and progesterone induce ductal elongation and branching during puberty, prolactin and related hormones prompt the proliferation and differentiation of the secretory epithelium during pregnancy. After the cessation of lactation, the secretory epithelium apoptoses and is remodeled, only to be rebuilt during the next pregnancy. Upon binding of prolactin to its receptor during pregnancy, the Jak2/Stat5 pathway is recruited, which in turn leads to the activation of a developmental program. Experimental genetics have demonstrated that the prolactin receptor is absolutely required for alveolar proliferation (Ormandy et al., 1997), that Stat5a is partially required for epithelial proliferation and differentiation (Liu et al., 1997) and that Stat5b can partially compensate for Stat5a (Liu et al., 1998). Since mice deficient for Stat5b alone or Stat5a/b are infertile (Teglund et al., 1998), it was not possible to determine the individual and combined contributions of these transcription factors during normal mammary development independent of an aberrant hormonal environment. We have now performed transplantation experiments and studied epithelial cells deficient in the prolactin receptor, Stat5a and Stat5b alone and Stat5a/b combined in the context of wild type mice in a physiological hormonal milieu. While the absence of Stat5a results in reduced epithelial proliferation and differentiation, virtually no alveolar epithelium developed in the absence of both Stat5a and 5b. Based on these data we propose that although Stat5a is vital for mammary function, Stat5b also contributes to the proliferation of alveolar epithelium. Furthermore, we provide evidence that prolactin mediated signaling in the mammary gland proceeds mainly, if not exclusively, through Stats 5a and 5b.

5. The Endocrine Society's 83rd Annual Meeting Denver, Colorado June 20-23, 2001

Using mouse knockout models to understand signal transduction pathways required for early mammary gland development

Sandra L. Grimm*, Keiko Miyoshi+, Lothar Hennighausen+, Chris Ormandy^, Teresa L. Wood#, Russ Hovey&, Barbara Vonderhaar& Jeffrey M. Rosen*

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The study of mouse knockout (KO) models has helped elucidate the *in vivo* function of many different genes involved in the various stages of mammary gland development. Previous analyses of C/EBPß-deficient mice have demonstrated an important role for this transcription factor in ductal morphogenesis and lobouloalveolar development of the mammary gland. In the pre-pubescent mouse, progesterone receptor (PR) expression is uniform along the ducts, but becomes non-uniform and scattered following the completion of ductal morphogenesis. However, PR remained uniformly expressed in the mammary gland in C/EBPß KO mice throughout this time frame. These changes were, however, not restricted to PR; similar increases in prolactin receptor (PrlR) mRNA and the estrogen receptor were also observed. When PR expression was examined in the PrlR KO gland, a uniform pattern of expression was also observed similar to the C/EBPß KO gland. In order to determine if these effects were epithelial cell autonomous and independent of any alterations in systemic hormone levels, PrlR KO and Stat5 A/B double KO mammary glands were transplanted into the cleared fat pads of nude mice. Quantitation of PR- and BrdU-positive cells in the glands of Stat5 A/B double KO transplants showed levels intermediate to that of wildtype and PrlR KO, suggesting that not all of the PrlR signaling is mediated through Stat5. Proliferating ductal cells rarely co-localized with steroid receptor-positive cells, supporting a paracrine mechanism of action. Interestingly, a decrease in both IGF-II and IGFBP-5 has also been observed in the C/EBPß KO mice. Together these data suggest that C/EBPß regulates mammary epithelial cell fate resulting in the correct spatial pattern of gene expression required to permit steroid hormone-regulated cell proliferation. (Supported by NIH grant CA 16303 and a DAMD fellowship #17-00-1-0138 to SLG)